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Design and prospective evaluation of a risk-based surveillance system for shrimp grow-out farms in northeast Brazil

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ABSTRACT

The farming of Pacific white shrimp *Litopennaeus vannamei* in northeast Brazil, has proven to be a promising sector. However, the farming of Pacific white shrimp in Brazil has been affected negatively by the occurrence of viral diseases, threatening this sector's expansion and sustainability. For this reason, the drafting of a surveillance system for early detection and definition of freedom from viral diseases, whose occurrence could result in high economic loses, is of the utmost importance. The stochastic model AquaVigil was implemented to prospectively evaluate different surveillance strategies to determine freedom from disease and identify the strategy with the lowest sampling efforts, making the best use of available resources through risk-based surveillance. The worked example presented was designed for regional application for the state of Ceará and can easily be applied to other Brazilian states. The AquaVigil model can analyse any risk-based surveillance system that considers a similar outline to the strategy here presented.

1. Introduction

The growing global demand of aquatic animals and aquatic animal products has led to high rates of production and trade frequency and increased concern over the occurrence and spread of viral diseases affecting various cultured species. As a result, many nations have adopted surveillance strategies to protect their aquaculture sector (FAO, 2014). The threat to aquaculture sustainability and safe international trade has also led countries to apply trade standards based on their own aquatic animal health status (FAO, 2014). When able to demonstrate that a particular disease agent is absent, a country can facilitate trade or apply import risk analysis (FAO, 2014; WTO, 2014).

The absence of infection, from here on referred to as freedom from disease, can be determined through the aggregation over time of negative outcomes generated from a surveillance system. Documenting freedom from disease requires a large sample frame and so surveillance activities should selectively target the high-risk strata of the population through risk-based surveillance (RBS) (Cameron, 2009). In this paper, a stochastic model based on scenario tree modelling was implemented to determine the probability of freedom obtained for certain surveillance efforts, so that the best strategy can be determined prospectively. The model outputs will provide decision makers with the information to implement surveillance efforts to achieve a desired probability of freedom. The surveillance system will also ensure early disease detection.

In northeast Brazil, the farming of Pacific white shrimp *Litopennaeus vannamei* has been an important source of income for large-scale producers and also the main or supplementary income-generating activity for the poorest rural communities and their small-scale farmers. In many cases, the occurrence of viral diseases has lead to the abandonment of farming activities (Ostrensky et al., 2008). There is to date, unclear knowledge on the geographic extent and impact to which viral diseases have affected the countries shrimp aquaculture sector. From the list of notifiable viral diseases in shrimp populations drafted by the Brazilian Ministry of Fisheries and Aquaculture (MPA), the Centre for Environment, Fisheries and Aquaculture Science (Cefas) references the presence of White Spot Syndrome Virus (WSSV), Infectious Myonecrosis Virus (IMNV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) and Taura Syndrome Virus (TSV), initially ruling out efforts for determining country-level freedom. Meanwhile, other

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viral pathogens such as Yellowhead Virus (YV) have yet to be identified at a national level (CEFAS, 2014; DOU, 2015).

The MPA has recognized the threat of viral diseases to the aquacultured shrimp populations and the need to strengthen disease surveillance (MPA, 2011). The needed surveillance system must encounter international acceptance and therefore follow World Organisation for Animal Health (OIE) surveillance guidelines (Corsin et al., 2009; OIE, 2014a). Implementing surveillance efforts to declare disease-free status can lead to early disease detection and identification of disease-free zones. The model can be applied to any one of the listed notifiable viral diseases, as the epidemiology, pathogenicity and many clinical features are similar among them.

2. Methods

2.1. Model overview

The AquaVigil model evaluates the results of implementing two types of surveillance system components that are activities that generate the needed information to determine freedom from disease: one active surveillance system component (ActiveSC) and one passive surveillance system component (PassiveSC). Surveillance system sensitivity (SSe), that is, the probability of the surveillance system detecting disease if it were present, can be estimated considering the joint contribution of the two surveillance components that make up the surveillance system: the PassiveSC sensitivity (SePassiveSC) and the ActiveSC sensitivity (SeActiveSC).

The model was developed in R environment and is available as the AquaVigil function in the Supplementary Document 1 with example data in the Supplementary Document 2 and using the mc2d, plyr, ggplot and Hmisc packages (R Development Core Team, 2008). The simulation comprised of 10,000 iterations and set a fixed random number seed for reproducible random results. Model inputs necessary for analysis are a comma-separated values (CSV) file with an ID column, four columns characterizing the presence (1) or absence (0) of four risk factors (RFs) and a fifth column specifying the number of samples retrieved from each farm. Prospectively, we can determine the SePassiveSC, the SeActiveSC, the SSe, the probability of freedom obtained through surveillance, the sample size for the ActiveSC and campaigns needed to achieve a desired probability of freedom. Other model outputs include a correlation analysis for surveillance system component sensitivities, the achieved probability of freedom after a single surveillance campaign and the sensitivity ratio for the ActiveSC (SR).

2.1.1. Data sources

A past census of the productive, technological, economical, social and environmental aspects of Brazil's aquaculture sector was drafted for the year of 2011 and the data provided by shrimp grow-out farms when questioned for this census was the data here used. From the available data, the worked example presented for determining disease freedom for the state of Ceará accounts for 325 grow-out farms, parameterized for the presence and absence of the selected RFs. The census data provided the coordinates for 273 of the 325 farms. To roughly illustrate the density of farmed areas, a map of such farms is provided in Fig. 1. From this map, the main farming areas are visible along the Jaguaribe River and delta, to the South, and the Acaraú River delta, to the North (ABCC/MPA, 2013).

2.1.2. States to define disease-free zones

Dispersion of infectious agents through water occurs frequently and at a rapid rate (Hoa et al., 2011; Lotz, 1997; Moss et al., 2012). This would strongly suggest the rapid spread of the pathogens between farms in interconnecting water systems. Therefore, the level at which disease freedom can be defined should be reasonably large. Furthermore, shrimp grow-out farms will frequently use post-larvae (PL) from PL suppliers in their state. Given the previous considerations, the state level was considered to define disease freedom. The state level will also allow a more efficient organizational approach to surveillance. A worked example of the AquaVigil model for Brazil's state of Ceará is presented, as this was determined as one of the leading states for aquacultured shrimp production (ABCC/MPA, 2013).

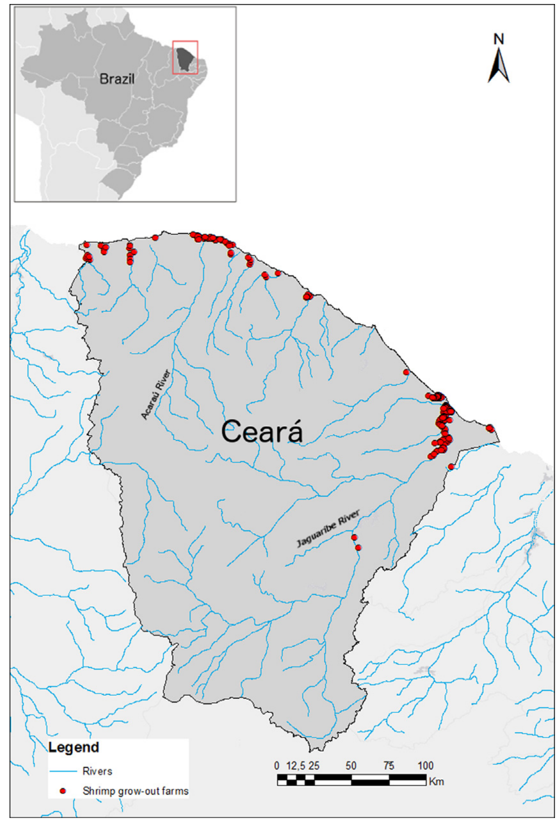

Fig. 1. Map of shrimp grow-out farms in the state of Ceará.

2.1.3. Surveillance of farmed populations

The "Report of the meeting of the Task Force on Animal Disease Surveillance Brussels, 24 and 25 June 2009" addressed how to demonstrate disease freedom from WSSV. The task force discussed how surveillance at the farm level would suffice when the disease is well known and the population in the farms is representative of both wild and farmed populations (European Commission, 2009). Taking into account the aforementioned conditions and the data available, only sampling of shrimp from grow-out farms was considered.

2.1.4. Time period for analysis

Australian authorities considered one campaign as sufficient to determine disease freedom for WSSV (East et al., 2004, 2005). Here we consider a single campaign both sufficient and most desirable to determine a cost-effective surveillance strategy, given the contribution of both surveillance system components. The water temperatures that could determine a higher probability of clinical signs of disease at certain time periods, are relatively constant for Brazil's northeast states, and so there is no time of year recommended to perform surveillance activities (Nunes et al., 2005). Therefore, the time periods for analysis of the surveillance system results will cover one year of surveillance.

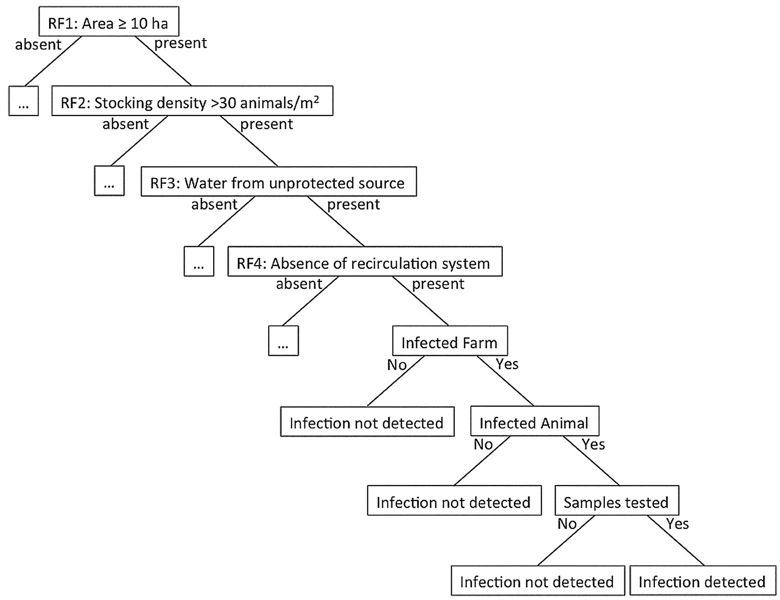

Fig. 2. The scenario tree for the active surveillance system component.

2.1.5. Design prevalence

In the absence of positive findings, the surveillance system can be applied to determine that disease is absent at a level equal to or greater than that of the design prevalence (Cameron, 2009). To incorporate the effect of *clustering*, two values of design prevalence can be used in the model: animal level design prevalence (Pu*) and farm level design prevalence (Ph*). For highly contagious diseases, such as those affecting species in an aquatic environment, a high proportion of infected animals are expected, and therefore, high values of design prevalence can be used (Martin et al., 2007). The OIE recommends the adoption of values of 1–5% for Pu* for slow-moving diseases and above 5% for highly contagious diseases, while values for Ph* should not exceed 2%, unless clearly justified (Corsin et al., 2009). However, published articles of Australian surveys, to determine country-level freedom from WSSV, used two sets of values for Ph* and Pu*: one where both took the fixed value of 10% and another where Ph* was set at 5% and Pu* was set at 10% (East et al., 2004, 2005). Keeping in mind the aforementioned values, for the worked example, the design prevalence was set at a value of 10% for Pu*, while the value of Ph* was kept conservatively lower, at 5%.

2.1.6. Test sensitivity

The OIE Manual of Diagnostic Tests for Aquatic Animals refers to molecular techniques, such as PCR followed by sequencing, for targeted surveillance to declare freedom for viral diseases affecting shrimp (Corsin et al., 2009; OIE, 2014b). The test sensitivity, a measure of the tests ability to identify truly infected animals, is considered to be high for tests based on molecular techniques (Corsin et al., 2009). A survey to determine disease-free status for WSSV in Australia considered a point value of 95% test sensitivity for the OIE recommended PCR protocol from Lo (1996) (East et al., 2005; OIE, 2014b). Consequently, these were the values considered for the test sensitivity for both initial PCR screening and confirmatory diagnosis through sequencing. The combined test sensitivity was set as the product of individual test sensitivities. Since follow-up testing to investigate true status of infection is always applied, the specificity of the testing protocol is considered to be 100%.

2.2. Active SSC

Active surveillance generates information on the health status of a population through the periodic collection of samples. In order to reduce the sample size needed to determine disease-free status, the ActiveSC will account for targeted sampling of grow-out farms at greatest risk of disease introduction, through RBS.

2.2.1. Scenario tree

A scenario tree illustrates the process by which the surveillance component can result in disease detection. In the scenario tree, factors affecting the probability that an individual unit or grouping level of units is infected are taken into account to determine the SeActiveSC. The SeActiveSC was determined using the methodology of Martin et al. (2007) based on the conceptual scenario tree in Fig. 2. For reasons of practicality, the scenario tree illustrates but one possible path to disease detection, considering high-risk farms those accounting for the presence of all RFs. Furthermore, the RFs are considered independent and their order unimportant in the scenario tree.

2.2.2. SeActiveSC

The relative risks (RRs) for the RFs represented as risk nodes in the scenario tree are adjusted according to Eqs. (1) and (2)) so that the average RR of a representative sample of the reference population in a risk node is 1, while maintaining a relativity specified in the inputs.

$$AR_1 = RR_1/(RR_1 * PropRR_1 + PropRR_0) \quad (1)$$

$$AR_0 = 1/(RR_1 * PropRR_1 + PropRR_0) \quad (2)$$

where AR_1 and AR_0 are the adjusted risks for the RRs presence (1) and absence (0) and $PropRR_1$ and $PropRR_0$ are the proportions of the reference population that fall into the two branches of the risk node. The AR values are used to determine the effective probability of a farm being infected (EPIH), if infection is present at Ph* (Eq. (3)).

$$EPIH_s = Ph^* * \prod_{r=1}^{R}(AR_r) \quad (3)$$

where R is the number of RFs and EPIH_s is determined for every s risk strata. The AquaVigil model accounts for four RFs, each with two possible outcomes, for a total of 16 (=2^4) risk strata. The unit sensitivity (SeU) is the probability that a single animal will give a positive result, if tested, and is set as the product of initial and confirmatory test sensitivities. When SeU is multiplied by the probability of a unit being infected, Pu^*, we have the probability of a positive test result for a single randomly selected unit. The grow-out farm level sensitivity (SeH) is the probability that infection will be detected at the farm level. For each sampled farm, a value of SeH is determined by the binomial method (Eq. (4)), as the number of shrimp sampled per farm will be lower than 10% of the total number of shrimp in a grow-out farm.

$$\mathrm{SeH} = 1 - (1 - \mathrm{SeU} * Pu^*)^n \tag{4}$$

where n is the number of animals sampled for each farm.

To determine the SeActiveSC, the probability of detecting infection at the state grouping level, the hypergeometric method is used since the number of sampled farms will be above 10% of the total number of farms in the state (Eq. (5)).

$$\mathrm{SeActiveSC} = 1 - \prod_{s=1}^{S} (1 - \mathrm{SeHav}_s * n_s/N_s)^{(N_s * \mathrm{EPIH}_s)} \tag{5}$$

for s risk strata, where:

- SeHav_s is the average SeH for the sth risk strata;
- n_s is the number of sampled farms in the sth risk strata;
- N_s is the number of farms in the population for the sth risk strata.

2.2.3. Sensitivity ratio

In order to compare the sensitivity of targeted risk-based sampling with equivalent random sampling, a SR of these values is returned as an output of the AquaVigil model to assess the effectiveness of the targeted surveillance approach. An equivalent random sample was considered a sample with the same number of farms and the same number of sampled animals per farm. A SR superior to 1 indicates a gain in sensitivity, while lower values reflect loss in sensitivity and values equal to one an equal sensitivity between sampling strategies.

2.2.4. Random sampling

The samples collected for RBS are compared to the needed samples size if performing representative random sampling of the population. The number of sampled farms ($N_{representative}$) (Eq. (6)) and animals ($n_{representative}$) (Eq. (7)) per farm for a representative random sample was determined using a two-stage sampling method (Cameron et al., 2014).

$$N_{representative} = (N/\mathrm{SeH}_{desired}) * \left(1 - (1 - \mathrm{SSe}_{desired})^{(1/(Ph^* * N))}\right) \tag{6}$$

$$n_{representative} = \ln(1 - \mathrm{SeH}_{desired})/\ln(1 - \mathrm{SeU} * Pu^*) \tag{7}$$

where:

- N is the number of farms in the state;
- $\mathrm{SeH}_{desired}$ is the desired farm level sensitivity;
- $\mathrm{SSe}_{desired}$ is the desired sensitivity for the surveillance system;
- Ph^* is the design prevalence at farm level;
- SeU is the product of diagnostic test sensitivities;
- Pu^* is the design prevalence at the animal level.

A desired system sensitivity of 95% and farm level sensitivity of 95% were considered, as these would be values commonly used for two-stage sampling to determining disease-free status in a single sampling campaign.

2.2.5. RFs for introduction of infection at the farm-level

Sampling high-risk farms aids early disease detection and lowers sampling efforts for surveillance. Scientifically documented RFs for introduction of shrimp viral diseases at grow-out farm level need to be identified to perform targeted sampling of the high-risk farm. However, in the absence of specific publications, RFs were selected from the data collected in the census data for 2011. The authors considered four RFs to be a reasonable number to allow the differentiation of a sufficient number of high-risk farms. The high-risk grow-out farms were considered those where all four RFs for the introduction of infection were present.

Farming intensities. When looking to enhance disease detection, one should consider the intensity of farming practices. Frequently, the intensity of farming can be inferred from both the size of the farms and the density of shrimp cultivated in the grow-out ponds. Larger farms are more likely to use large volumes of non-treated water, receive numerous shipments of PL to stock the farms and are subject to an increased movement of people, vehicles and animals, which can serve as pathways for disease introduction (Lightner, 2005; Lotz, 1997). Therefore, the RF characterizing the type of grow-out farm in terms of production was considered. This RF was present for large to medium scale grow-out farms, as the RF "cultivating areas equal to or above 10 hectares", absent for micro and small producers, with lower cultivating areas.

Shrimp grow-out farms also differ in the densities of shrimp cultivated per square meter. Farms in Ceará apply varying stocking densities, irrespectively of farm size, increasing intensity of shipments of PL onto the farm and increasing the risk of disease introduction. Furthermore, applying high stocking densities to production systems without the proper management can lead to increased stressing of the shrimp and risk of disease occurrence (Kautsky et al., 2000). A second RF indicating the high density of shrimp is therefore considered present as the RF "densities above 30 or more shrimp per square meter" are stocked, absent when stocking densities are equal to or below this value. The values for the aforementioned RFs were chosen as they are used in Brazil to distinguish small and medium-scale farms ABCC/MPA (2013).

Biosecurity. Biosecurity measures are implemented at the aquaculture establishment to reduce the likelihood of introduction of infectious pathogens (Lotz, 1997). Therefore, biosecurity measures are important when determining which RFs to include in the model. Pathogen exclusion is frequently accomplished by stocking farms with controlled water sources and disease-free shrimp (Lightner, 2005). The importance of stocking farms with PL free from disease, either certified through rigorous testing or supplied as specific pathogen free PL (SPF) is central to biosecurity in shrimp aquaculture (Bray et al., 2004; Clifford and Cook, 2002; Corsin et al., 2005; Hoa et al., 2005; Lightner, 2003, 2005; Lotz, 1997; Walker et al., 2011). However, information on the use of SPF PL was not available from the census that supplied the data for analysis. On the other hand, the census did provide information on the main source of water supplied to the farm. The water used for shrimp farming is considered as one of the most significant ways of pathogens introduction at the farm level (Moss et al., 2012). A third RF was therefore considered, the use of "water from unprotected source", where water supplied from a source other than a well is regarded as a source of pathogen introduction. Limiting pathogen introduction can also be accomplished through "zero" water exchange (Lightner, 2005). A biosecure grow-out farm will limit water exchange by means of a recirculating system (Lotz, 1997). Consequently, a fourth RF was considered, the "absence of a recirculation system". Since there is no true "zero" water exchange and water can be supplied from an unprotected source, these RFs are not considered dependent nor redundant. Once more, the census provided information on the water source and use of a recirculation system ABCC/MPA (2013).

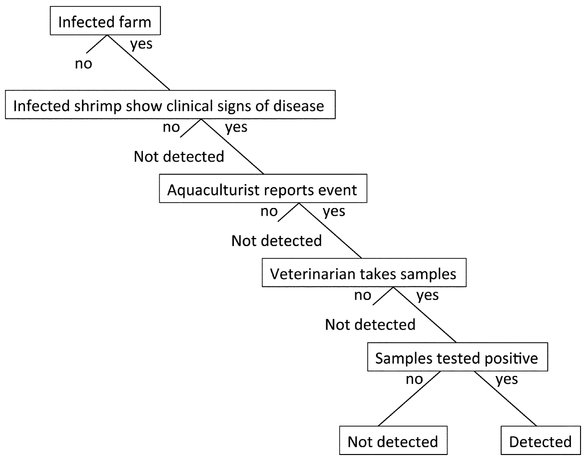

Fig. 3. The scenario tree for the passive surveillance system component.

2.2.6. Relative risks

The accuracy of the model depends heavily on the values chosen for this key model parameter. This is among one of the recognizable difficulties that currently limit the developing of RBS in the aquatic context. A common approach to estimating such parameters is the use of information from expert panels. Experts in the field of shrimp health in Brazil that could provide adequate estimates are scarce. Furthermore, the reliability of any estimates that could be provided would be greatly uncertain, given the present knowledge of occurrence of shrimp diseases in Brazil. The still-developing field of aquatic animal surveillance and specific epidemiological studies also made literary references to support the choice of values unattainable (Oidtmann et al., 2013; Peeler and Taylor, 2011).

Given the previous considerations, values for RR were assigned arbitrarily in a conservative way that would reflect the underestimation of the importance of any RF. The RFs were equally weighed and considered to have two mutually exclusive outcomes: presence or absence. When present, all RFs were parameterized with a value of RR modelled in R environment as Pert distributions with minimum values of 1, most likely values of 2 and maximum values of 3. These values translate into a minimum RR of one, a most-likely value of twice the risk of disease introduction for exposure to the RF and a maximum value of 3 times the risk to obtain a symmetrical Pert distribution. Where the RF was absent, RR was appointed a fixed value of 1.

2.3. Passive SSC

Passive surveillance is implemented for early detection of disease and can contribute to determining disease free status over time. Passive surveillance allows for the comprehensive coverage of the shrimp grow-out farm population, as all units are potentially subject to surveillance. The unit of analysis for the SePassiveSC are the shrimp grow-out farms.

2.3.1. Scenario tree

Detecting disease through passive surveillance relies on the probability that, at the farm level, infected animals show clinical signs of disease, that aquaculturists are aware of these signs and are motivated to report a suspected disease occurrence, that state veterinary authorities investigate the event and correctly suspect the occurrence of a specific viral disease and also on the diagnostic capacity of the tests to detect and confirm the presence of infection (Hadorn and Stark, 2008). The SePassiveSSC is determined according to Martin et al. (2007). The scenario tree illustrating the detection process for the PassiveSC is illustrated in Fig. 3.

Table 1
Parameters for three levels of probability of disease awareness: low, medium and high.

Probability of detection	Minimum	Most likely	Maximum
Pert distribution			
Low	0.1	0.2	0.3
Medium	0.4	0.5	0.6
High	0.7	0.8	0.9

2.3.2. SePassiveSC

A simple approach was considered to calculate the SePassiveSC. The unit sensitivity for the PassiveSC (SeHP), that is, the probability that any randomly selected farm will give a positive test result, considers the probability that infection will result in shrimp developing clinical signs of disease (PRClinicalSigns), the probability of detection by aquaculturist (PrAquaculturist) and the probability of further investigation by a veterinarian (PrVeterinarian), along with the diagnostic test sensitivities (SeTests) and the number of $n_{passive}$ farms tested from the N farms in the population (Eq. (8)) (E. Sergeant, personal communication, May 28, 2015). We consider that the shrimp sent for testing are infected and so the probability $P_{sampled\,fish\,infected}$ was set at 1. For the present example, we considered the PassiveSC would sample 10 farms ($n_{passive}$).

$$\mathrm{SeHP} = Pr\,Clinical\,Signs * Pr\,Aquaculturist * Pr\,Veterinarian * (1 - (1 - P_{sampled\,fish\,infected} * SeTests)^{n_{passive}}) \quad (8)$$

The SePassiveSC can then be determined through Eq. ((9)) (E. Sergeant, personal communication, May 28, 2015).

$$\mathrm{SePassiveSC} = 1 - (1 - \mathrm{SeHP})^{(Ph^{*} * N)} \quad (9)$$

2.3.3. PassiveSC probabilities

The probabilities of the aquaculturist detecting symptoms of disease and contacting state veterinary authorities, and that state veterinary authorities suspect disease and conduct further investigation by sending samples for testing, can be considered for varying levels of disease awareness. These levels of varying awareness were parameterized according to Hadorn and Stark (2008) for three levels of low, medium and high disease awareness (Table 1). The level considered for analysis to conservatively represent the present level of awareness, is the lowest of these levels. Incrementing disease awareness may increase the SePassiveSC and can also be determined through the AquaVigil model.

The probability that animals show clinical signs of disease is difficult to determine for the listed notifiable viral shrimp diseases. Evidence suggests that long-term viral infections, such as WSSV, can be present without producing clinical signs of disease and disease outbreaks (Tsai et al., 1999). For this reason, the authors determined that a conservatively wide range of values should parameterize the Pert distribution for the probability of shrimp showing clinical signs of any listed viral disease, and so a minimum value of 1%, a most likely value of 5% and a maximum value of 10% were chosen.

2.4. Surveillance system sensitivity

The overall SSe of the AquaVigil model worked example for the state of Ceará was determined assuming the independence of the active and passive surveillance components (Eq. (10)) (Cameron, 2009).

$$\mathrm{SSe} = 1 - (1 - \mathrm{SeActiveSSC}) * (1 - \mathrm{SePassiveSSC}) \quad (10)$$

2.5. Probability of freedom

The probability of freedom can be determined at the end of a surveillance period, when evaluating the information gathered through the surveillance components. The method to determine the probability of freedom also followed Martin et al. (2007). Using Bayes' theorem, the probability of the state being free from disease depends on the value of SSe and the prior probability that disease is present before the surveillance efforts are undergone. The authors selected an initial prior for the probability of freedom parameterized as an uninformed prior with a value of 0.5, for a 50% probability of disease presence or absence, following the example from terrestrial animal surveillance systems for disease freedom by Martin (2008) and More et al. (2009). It is important to mention that the aforementioned value, chosen by the authors in the absence of surveillance data, can inevitably bias disease freedom conclusions (Gustafson et al., 2010).

The AquaVigil model outputs the number of campaigns needed to achieve a desired probability of freedom when the same surveillance sampling strategy is repeatedly applied for active and passive surveillance, over what may be many periods of time. The probability of freedom is determined at the end of every surveillance campaign, in most cases, increasing the probability that the disease will be absent at the beginning of the next surveillance time period (TP). Considering the SSe and prior probability of freedom for the time period $Prior_{TP}$, a posterior value of probability of freedom (PostPrFree) is determined from Eq. ((11)) (Martin et al., 2007).

$$\text{PostPrFree}_{TP} = (1 - Prior_{TP})/(1 - Prior_{TP} * \text{SSe}_{TP}) \quad (11)$$

There is a need to consider that, during a time period, disease may be introduced into the state and so the value of PostPrFree is adjusted. In Brazil, import risk analysis minimizes the probability of disease entering the country (PrIntro), as does the significant geographic distance from other South American shrimp producing countries. In Ceará, the main route for disease introduction is through the uncontrolled movement of crustaceans and through the oceanic currents from neighbouring states. To account for the possibility of introduction of disease, the PrIntro was parameterized by the authors as a Pert distribution of minimum 1%, most likely 1,5% and maximum 2%, for a conservatively high estimate when compared to the values used for land-animal production systems, such as the point estimate in Frossling et al. (2009) of 0.833%. The value of $\text{PostPrFree}_{adjusted}$ is determined from the value of posterior probability of infection ($\text{PostPrInf}_{adjusted}$) (Eq. (12)) in Eq. ((13)) (Cameron, 2009).

$$\text{PostPrInf}_{adjusted} = \text{PostPrInf} + \text{PrIntro} - \text{Post}PrInf * \text{PrIntro} \quad (12)$$

$$\text{PostPrFree}_{adjusted} = 1 - \text{PostPrInf}_{adjusted} \quad (13)$$

2.6. Relationship between variables

The model output included a sensitivity analysis between RFs and the SeActiveSC and between the probabilities considered for the passive component and the value of SePassiveSC. Spearman rank-order correlation coefficients can identify which RFs and probabilities are most correlated to the values of both SeActiveSC and SePassiveSC. The model returns the values of the Spearman's correlation coefficient as a tornado plot. Values close to −1 and 1 indicate that the variables are highly negatively and positively correlated, respectively. The Spearman's correlation coefficients are determined and tested for statistical significance by constructing a test statistic t, where statistically significant Spearman rank-order correlations have an associated p-value of less than 5%. Correlation coefficients and significance test are determined through the Hmisc package (Harrell Jr., 2015).

Table 2
Scenario 1 sampling strategies to reach a probability of freedom superior or equal to 94.5% for the state of Ceará.

Sampling frame		Required sampling	
High-risk farms	Animals per farm	Campaigns	Total samples
50	32	1	1600
45	32	1	1440
40	32	1	1280
35	32	1	1120
30	32	1	960
25	32	1	800
20	32	2	1280

3. Results and discussion

3.1. Best sampling strategy for high-risk farm sampling

The sampling strategy for RBS considered the exclusive targeted sampling of high-risk grow-out farms, and therefore, farms with all four RFs. From the 325 grow-out farms in Ceará, 50 farms are characterized by the presence of all four RFs. Lower numbers of farms were sampled from then on, for 45, 40, 35, 30, 25 and 20 high-risk farms, to identify a more practical and cost-effective surveillance strategy. The number of animals to sample from each farm for representative random sampling is 32 and this was the value chosen for analysis.

The PassiveSC can contribute to increase SSe and influence the total number of campaigns needed to achieve the desired probability of freedom. Therefore, we can determine how many sampling campaigns and animals are needed for sampling with or without the contribution from this type of surveillance activity. Furthermore, we can consider the contribution from this surveillance component when awareness is high among farmers and veterinarians. Therefore, the number of campaigns, animals sampled and the surveillance system performance can be determined for three scenarios:

- Scenario 1: implementing only the ActiveSC.
- Scenario 2: implementing the ActiveSC and the PassiveSC with low disease awareness.
- Scenario 3: implementing the ActiveSC and the PassiveSC with high disease awareness.

3.1.1. Scenario 1

For scenario 1, the best sampling strategy for targeted sampling is that where 25 high-risk farms are sampled, for a total of 800 sampled animals and a single surveillance campaign (Table 2). Random and representative two-stage sampling of Ceará's 325 farms, would require 1856 animals to be sampled from 58 farms, sampling 32 animals per farm, to declare disease free status with a single surveillance campaign. Applying the previous targeted sampling strategy would account for a reduction of 56.9% in number of farms and of animals needed for testing to declare freedom from any one of the listed notifiable viral diseases. Furthermore, if pooled sampling of 5 animals were considered, the overall number of diagnostic tests needed to declare freedom would fall from 800 to 160. However, if pooling of samples were to be done, as is frequent in aquatic animal diagnostics, PCR test performance could be affected and values for test sensitivity and specificity reconsidered. However, examples of surveys to determine disease freedom consider that the sensitivity of pooled testing is high, surpassing the value here chosen (OIE, 2014a).

If the surveillance strategy were to privilege a lower sample size per campaign and active surveillance for a two-year period, the best strategy is the sampling of 20 high-risk farms, for a total of 1280 samples, collecting 640 animals per campaign.

Table 3
Scenario 2 surveillance system component sensitivities, overall SSes and SRs for surveillance sampling frames analysed for the state of Ceará, with corresponding 2.5%tiles and 97.5%tiles.

Sampling Frame		Surveillance system performance			
High-risk farms	Animals per farm	SeActiveSC	SePassiveSC	SSe	SR
50	32	100.0% (100.0%; 100.0%)	3.10% (1.10%; 6.50%)	100.0% (100.0%; 100.0%)	1.06
45	32	100.0% (100.0%; 100.0%)	3.10% (1.10%; 6.50%)	100.0% (100.0%; 100.0%)	1.08
40	32	99.94% (99.66%; 99.97%)	3.10% (1.10%; 6.50%)	99.94% (99.67%; 99.99%)	1.10
35	32	99.66% (98.73%; 99.89%)	3.10% (1.10%; 6.50%)	99.67% (98.77%; 99.90%)	1.15
30	32	98.76% (96.55%; 99.49%)	3.10% (1.10%; 6.50%)	98.80% (96.65%; 99.51%)	1.22
25	32	96.50% (92.34%; 98.22%)	3.10% (1.10%; 6.50%)	96.62% (92.57%; 98.28%)	1.30
20	32	91.69% (85.13%; 94.95%)	3.10% (1.10%; 6.50%)	91.96% (85.57%; 95.13%)	1.37

Table 4
Scenario 3 surveillance system component sensitivities, overall SSes and SRs for surveillance sampling frames analysed for the state of Ceará, with corresponding 2.5% tiles and 97.5% tiles.

Sampling Frame		Surveillance system performance			
High-risk farms	Animals per farm	SeActiveSC	SePassiveSC	SSe	SR
50	32	100.0% (100.0%; 100.0%)	41.64% (19.26%; 60.28%)	100.0% (100.0%; 100.0%)	1.06
45	32	100.0% (100.0%; 100.0%)	41.64% (19.26%; 60.28%)	100.0% (99.97%; 100.0%)	1.08
40	32	99.94% (99.66%; 99.97%)	41.64% (19.26%; 60.28%)	99.97% (99.79%; 99.99%)	1.10
35	32	99.66% (98.73%; 99.89%)	41.64% (19.26%; 60.28%)	99.81% (99.20%; 99.94%)	1.15
30	32	98.76% (96.55%; 99.49%)	41.64% (19.26%; 60.28%)	99.29% (97.82%; 99.73%)	1.22
25	32	96.50% (92.34%; 98.22%)	41.64% (19.26%; 60.28%)	97.97% (95.08%; 99.09%)	1.30
20	32	91.69% (85.13%; 94.95%)	41.64% (19.26%; 60.28%)	95.17% (90.22%; 97.51%)	1.37

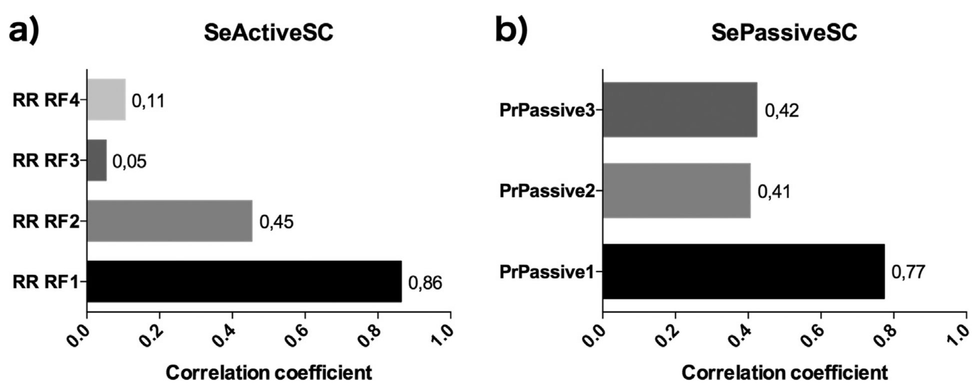

Fig. 4. Tornado plots of Spearman correlation coefficients to identify the variables most correlated with the values of SeActiveSC (a) and SePassiveSC (b).

3.1.2. Scenario 2

We can consider the contribution of the SePassiveSC to determine the number of campaigns and samples needed to determine disease free status. Here we conservatively estimated that 10 farms could have samples submitted for diagnostic exams through the PassiveSC. We verified that, for Scenario 2, where the level of awareness is low among the farmers and veterinarians, the PassiveSC does not contribute to increase the value of the SSe to the point of changing the number of samples or campaigns needed to establish disease free status. The sampling frame and number of campaigns to determine disease free status is the same as that presented for scenario 1 and so is the best sampling strategy. The values for SeActiveSC and SePassiveSC, overall SSe and SR for Scenario 2 are summarized in Table 3.

3.1.3. Scenario 3

For Scenario 3 we consider the increase of the level of disease awareness. There is an increase in SePassive contributing to the increase in overall SSe (Table 4). However, the increase in overall SSe does not translate into a change in number of samples required to determine freedom from disease in comparison to the previous scenarios.

3.2. SR and relationship between variables

All SR values are above one, indicating a gain of sensitivity when performing RBS compared to an equivalent random sampling.

The AquaVigil model output included tornado plots of Spearman correlation coefficients for sensitivity analysis that identified the inputs most correlated to the values of SePassiveSC and SeActiveSC. For the chosen surveillance strategy rendering the smallest sample size and low awareness, the RF with the greatest correlation relationship to the value of SeActiveSC was RF1, the aquaculture grow-out farms with cultivating areas above 10 ha. The sensitivity analysis, given the same surveillance scenario, for the SePassiveSC, identified the probability of infected animals showing clinical signs of disease as the input variable with the greatest correlation relationship for this value (Fig. 4).

4. Conclusion

The prospective evaluation of different surveillance strategies allowed the identification of the best strategy to declare freedom from any listed notifiable viral disease affecting shrimp aquaculture grow-out farms for the state of Ceará. The AquaVigil model took into account four RFs, among a wide range of other model parameters, returning the surveillance system performance before its application. The arbitrarity behind the choice of important model parameters inevitably alters the accuracy of the model. However, through their conservative estimation, the model can make disease freedom declaration a reality in the present context. Furthermore, it is important to state that the parameters can be changed as seen fit to evaluate different surveillance scenarios, as changes in any future context can occur and specific information made available.

This model provides decision makers with a tool for strategical planning of future surveillance activities capable of early detection of disease and, in the event of sampling efforts failing to detect disease, to declare disease freedom. The model can also be applied to evaluate real data from the surveillance system once implemented, when a single sampling campaign is deemed sufficient to determine disease-free status or when the sampling protocol is repeated for consecutive surveillance campaigns. The AquaVigil model can evaluate other surveillance strategies for varying production systems, diseases and surveillance scenarios, provided they follow a similar surveillance outline.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2015.10.022.

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